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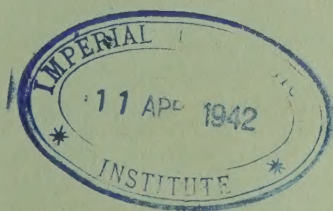
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No. 2

THE RELATIONSHIPS OF THE HEPATICAE

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The Hepaticae, as usually understood, comprise four orders: Anthocerotales, Sphaerocarpaceae, Merchantiales and Jungermanniales.

The Anthocerotales, however, while probably related distantly to the other Hepaticae, differ so greatly in the structure of both gametophyte and sporophyte that their separation, as a class, Anthocerotes, seems amply justified.

The single family, Anthocerotaceae, is a very natural one, but its relationships with the other Archegoniates are by no means clear and its relations to the other Bryophytes are very obscure. There are four genera, *Anthoceros*, *Megaceros*, *Dendroceros* and *Nothothylas*. The largest genus, *Anthoceros*, has been separated into two by Stephani. There are over 200 described species in the family, wide spread in the warmer parts of the world.

The gametophyte is a prostrate thallus, composed of nearly uniform cells, in most cases containing a single chromatophore, which closely resembles that of the Ulothricales and often contains a conspicuous pyrenoid. This alga-like chromatophore is not known elsewhere among the Archegoniates.

While the gametophyte of the Anthocerotaceae most nearly resembles the green algae from which it is believed the higher plants have been derived, the sporophyte is much better developed than in any of the Hepaticae, and is in some respects more like that of the mosses, or even the most primitive of the pteridophytes. Thus, the Anthocerotes may be said to form a synthetic, and presumably, very ancient group of plants with relationships on the one hand with the bryophytes and pteridophytes and, on the other, with the green algae.

It is not impossible that from the same stock as the Anthocerotes, the Hepaticae have been derived. Sometimes, as in *Megaceros*, the single chromatophore is replaced by several, and may be compared with those of some Hepaticae, like *Cyathodium*, for example. In Anthocerotes, unlike the other liverworts, the sporophyte is not only a spore-producing structure, but the sporogenous tissue, especially in the higher forms, like *Anthoceros*, is subordinated to the vegetative tissues. The sporophyte may live for months after the first spores are discharged, and the presence of a zone of meristematic tissue at the base enables it to increase in size, developing both new sporogenous and vegetative tissues. Abundant chlorophyllous tissue is present, and in some cases an efficient water-conducting tissue—thus approaching the independent condition found in the pteridophytes (1, 7, 12). A comparison has also been made between the sporophyte of *Notothylas*, the least specialized of the Anthocerotaceae, and that of *Cyathodium* or *Sphaerocarpus*, two of the simplest Hepaticae. It has been held that the Anthocerotes were derived from such simple Hepaticae through forms like *Notothylas*. It may be said, however, that admitting a real relationship between the Anthocerotes and Hepaticae, it seems more likely that the Anthocerotes are the older types and the Hepaticae the derivative ones. The sporophyte of the first Anthocerotes must have been much simpler than that of any of the existing forms—perhaps comparable to that of such liverworts as *Riccia* or *Sphaerocarpus*.

Excluding the Anthocerotes, the remaining liverworts show sufficient evidences of relationships among themselves to warrant, for the present at least, their inclusion in a single class, Hepaticae.

While there is good reason to assume that some of the Hepaticae are very old types, very little is known at present of their geological history, and the few known palaeozoic fossils are referable to existing types (32, 33). While certain groups are sufficiently well defined, *e.g.*, Sphaerocarpaceae, Ricciaceae, the relationships of these groups to each other, and the interrelationships of the members of the larger orders, are not always so evident, and it can hardly be said that an entirely satisfactory classification of the Hepaticae has been established. Three orders, Sphaerocarpaceae, Marchantiales and Jungermanniales, may be recognized (14) and possibly a fourth, Calobryales, may be added (11). *Calobryum*, the type

of the Calobryaceae, has been referred to the Jungermanniales, but differs essentially from the latter in the character of both the sexual organs and the sporophyte.

The simplest gametophytes of the Hepaticae closely resemble that of *Anthoceros*, a prostrate thallus composed of similar cells throughout, e.g., *Aneura*, *Pellia*. From this undifferentiated thallus there have been developed several specialized types, evidently arising independently in a number of divergent lines of evolution. In the Jungermanniales one type, e.g., *Metzgeria*, *Pallavicinia*, there is a definite midrib which may have a central strand of elongated conducting cells, sometimes with thickened cell walls, recalling the tracheary tissue of vascular plants. The lateral wings of the thallus consist of a single layer of chlorophyllous cells. Sometimes, e.g., in species of *Pallavicinia* and *Hymenophyton* (*Umbraclum*), there is a differentiation into a prostrate cylindrical rhizome-like portion from which upright branches arise, undergoing repeated dichotomy so that these upright shoots resemble the palmate fronds of a filmy fern. In some species of *Aneura* there is a central axis with numerous lateral branches—thus suggesting a pinnate fern-frond.

In another category are the leaf-like lobes found in a number of liverworts belonging to quite unrelated families. These lobes may be only slightly developed, e.g., in *Blasia* and some species of *Pallavicinia*, or they may develop into definite leaves, as in *Treubia* and *Noteroclada* whose leaves bear a definite relation to the apical cell of the shoot, as they do in the leaves of the acrogynous Hepaticae, the "foliose" or leafy liverworts. In most of the latter, the leaves form three definite series, corresponding to the segments cut off from the tetrahedral apical cell. The leaves in these Acrogynae are often complicated in structure.

The evolution of the gametophyte has been quite different in the Marchantiales. The strictly thallose form has been retained, but there has been a very marked differentiation of the tissues, culminating in such highly specialized types as the Marchantiaceae, e.g., *Marchantia*, *Fegatella*. The most primitive condition is found in some species of *Riccia*. In *R. glauca* the dorsal tissue of the thallus develops a system of narrow air-chambers opening at the surface and surrounded by the chlorophyllous cells which are in vertical rows. The ventral region is composed of compact tissue

which passes gradually into the green dorsal tissue. In the more specialized Marchantiaceae there is a definite epidermis with characteristic pores communicating with a system of air-chambers to which the green tissue is confined. In *Marchantia* and *Fegatella* these air-chambers form a single layer, sharply separated from the solid ventral tissue. Each air-chamber has a single large pore.

In some Hepaticae there is a preliminary structure, the "protonema," developed from the germinating spore, and the definitive gametophyte arises as a bud or branch from the protonema.

The sexual organs, archegonia and antheridia, may develop directly from the thallus, or there may be special receptacles developed upon which these are borne in the higher Marchantiales, like *Marchantia* and *Dumortiera*. The Hepaticae present a strong contrast to the Anthocerotales, in the variety shown by the gametophyte and the relatively highly specialized structures shown by some of them. On the other hand, the sporophyte is much less developed than that of the Anthocerotaceae and remains to a great extent parasitic upon the gametophyte. In its simplest form, *e.g.*, *Riccia*, it is merely a capsule with a single layer of wall cells enclosing a mass of spores. In the most highly developed forms, it shows an elongated stalk (seta) bearing the globular or cylindrical capsule. In addition to the spores there are the sterile cells, elaters. Practically no chlorophyll is present and the developing sporophyte is dependent upon the gametophyte for its growth. With discharge of the spores the tissues collapse and wither away. It thus offers a marked contrast to the long-lived and nearly self-supporting sporophyte of *Anthoceros*.

SPHAEROCARPALES

This small order, containing three genera and about 20 species is, on the whole, the simplest of the Hepaticae. The type genus *Sphaerocarpus* has several species on the Pacific Coast and in the southeastern states. *Sphaerocarpus* is dioecious, the males being much smaller than the females. The gametophyte is a simple thallus, composed of uniform cells, the central portion forming an indefinite broad midrib which merges gradually into the lateral wings composed of a single layer of cells. The sexual organs, each enclosed in a conspicuous involucre, cover the dorsal surface of the thallus.

The sporophyte is intermediate in structure between that of *Riccia* and that of the typical Marchantiales. Of the two cells resulting from the first division of the zygote, the upper (epibasal) cell gives rise to the globular capsule, the lower (hypobasal) to the haustorium (foot). Unlike *Riccia*, where all of the inner cells of the capsule produce spores, in *Sphaerocarpus* some of the sporogenous cells remain undivided, but do not develop into the elaters of the typical liverwort sporogonium.

The second genus of the Sphaerocarpaceae consists of *Geothallus tuberosus* (5), a monotypic genus from southern California, differing from *Sphaerocarpus* in its much larger size and the development of definite leaves, much like those of *Fossombronia*, one of the Jungermanniales, with which the Sphaerocarpaceae were formerly associated. The third genus, *Riella*, while agreeing with *Sphaerocarpus* in the structure of the sexual organs and sporophyte, differs greatly in its habit, being a submersed aquatic. Most of the species of *Riella* occur in the regions adjacent to the Mediterranean, but one occurs in the United States, and others in the Canary Islands, and South Africa.

While the Sphaerocarpaceae were formerly associated with the Jungermanniales, they were later separated (14) as an order coordinate with the Marchantiales and Jungermanniales, and to some extent intermediate between them.

MARCHANTIALES

The Marchantiales, comprising about 400 species, form a very clearly defined order. Most of the genera and several species are cosmopolitan.

The gametophyte is always a prostrate thallus, commonly branching dichotomously, and there is no development of leaf-like photosynthetic organs so characteristic of the more specialized Jungermanniales. The thallus, however, in most of them, shows a remarkable degree of differentiation of the tissues. Usually the massive thallus has the ventral region composed of compact tissue with little or no chlorophyll present, while the green tissue is confined to the dorsal region. In the less specialized genera, like *Riccia*, there is a transition from the green dorsal tissue to the colorless ventral region. In highly specialized genera, as *Marchantia* and *Fegatella*, the chlorophyllous tissue is sharply segre-

gated and there is a single tier of dorsal air-chambers, or lacunae, which open at the surface through characteristic pores (stomata) in the epidermis. The green tissue occupies the floor of the air-chambers from which short rows of green cells extend into the air-chamber and thus form a very efficient photosynthetic apparatus.

Another and less specialized type is found in some other genera, e.g., *Fimbriaria*, where the lacunae are large and irregular in form and the lacunar region is not clearly delimited from the compact ventral tissues.

The genera *Monoselenium*, *Dumortiera* and *Monoclea* differ from the typical Marchantiales in having the thallus, like that of *Anthoceros* or *Aneura*, composed of uniform green tissue with no lacunae. In *Monoclea* and *Monoselenium* there is no trace of the air-chambers but in some forms of *Dumortiera* there are evident remains of dorsal air-chambers which become almost completely obliterated. It is generally assumed that these genera are not primitive but have been derived from forms which possessed such air-chambers.

The Marchantiales are characterized by the presence of membranaceous scales developed on the ventral surface of the thallus. These scales are usually in two rows. Some of the rhizoids have thickened cell walls with conspicuous spike-like protuberances on the inner surface.

THE SEX ORGANS

The archegonia and antheridia are much alike in all the Marchantiales. The archegonium has six peripheral rows of neck cells, and the antheridium has a short pedicel. In *Riccia*, the archegonia and antheridia are more or less mixed, and are formed directly from the superficial cells of the thallus. In the most specialized genera, like *Marchantia*, the plants are dioecious and the reproductive organs are formed in greatly modified receptacles, borne on slender pedicels. There are various intermediate conditions between *Riccia* and the higher Marchantiaceae. Among these, the small family Corsiniaceae with the genera *Corsinia* and *Boschia*, may be said to connect the Ricciaceae and Marchantiaceae. In the latter, the female receptacle, or carpocephalum, is formed at the apex of the thallus by a rapid dichotomy of the apex and is, there-

fore, a compact branch system, each apex developing one or more archegonia.

In none of the Marchantiales does the sporophyte attain a degree of specialization corresponding to that of the gametophytic structures. In all of them the wall of the capsule consists of but a single layer of cells, except that in some cases there is a more or less thickened cap, by which the capsule opens; but as a rule the capsule wall breaks irregularly. Except in the Ricciaceae and Corsiniaceae, elaters are formed and there is a short seta and foot. The three families, Ricciaceae, Corsiniaceae and Marchantiaceae form an interesting series, showing the progressive evolution of the sporogenous tissue (26).

Besides these three families there are two others whose relation to these and to each other are not so clear. They probably represent two independent lines of development originating near the base of the Marchantiales. It has been suggested that they are connected with the Ricciaceae through the Corsiniaceae, but it is not impossible that they may have been derived from forms more nearly related to the Sphaerocarpales. The Targioniaceae have two genera, *Targionia* and *Cyathodium*. The only American representative is *Targionia hypophylla*, common on the Pacific Coast but not found in eastern North America. This species is widely distributed, occurring also in Europe, South Africa and Australia. The structure of the thallus is much like that of the Marchantiaceae but no carpocephalum is developed.

Cyathodium has about a half-dozen species in moist tropical regions, growing in shady locations, like the openings of caves. The thallus is very delicate in texture and the chloroplasts are large and few in number.

MONOCLEACEAE (4, 14, 20, 29)

This small family contains but two species—*Monoclea Forsteri* of New Zealand and Patagonia, and *M. Gottschei* from tropical America. There is much difference of opinion as to the systematic position of *Monoclea* but the weight of evidence indicates that it should be included in the Marchantiales.

In general appearance the gametophyte resembles a large *Anthoceros* or *Aneura*, the thallus being composed of uniform solid tissue with no trace of the characteristic air-chambers of typical

Marchantiales. Moreover, the sporophyte has a very long seta, like that of most Jungermanniales. However, development of the sexual organs and the sporophyte conform to the type of Marchantiales rather than to that of Jungermanniales (20). The lack of air-chambers is found also in *Dumortiera*, whose relationship with the higher Marchantiaceae is beyond question. *Monoclea Forsteri* often grows actually partially submersed and it may be assumed that the absence of air-chambers may perhaps be associated with this hygrophilous habit. Possibly the Monocleaceae should be placed at the beginning of the Marchantiales, near the point where the Marchantiales and Jungermanniales diverged from some common stock.

JUNGERMANNIALES

A recent enumeration of the Hepaticae shows that of a total of 8538 species, 7803 belong to the Jungermanniales. The order seems to be a natural one but the further classification is in a very unsatisfactory condition. There is much difference of opinion as to the limits of the families and the genera belonging to them. The classification has been based largely upon external characters and only a relatively small number of species has been studied critically as to their life-history and, especially, the development of the sporophyte. Until much more has been done in this direction, any proposed classification must remain to a great extent merely tentative.

Of the classifications that have been proposed, perhaps that of Cavers (14) is the most satisfactory. It follows, in the main, that of Schiffner (28) but differs in some important respects. To quote Cavers: "The Jungermanniales form a single phylum, the boundaries between the systematic families in most cases badly defined, and probably in no other group of plants do we find such striking and abundant examples of parallelism or homoplasy."

The gametophyte may be, on the one hand, a simple prostrate thallus, while with most specialized types there is a definite axis bearing leaves which have a direct relation to the segments of the apical cell. Between these extremes are many intermediate conditions, and it is evident that very similar structures have arisen in several independent lines of development. The tissues in the great majority of the Jungermanniales are very uniform and there is

nothing comparable to the highly specialized tissues of the gametophyte in the higher Marchantiales.

The sporophyte has a definite capsule and seta—the latter often much elongated—and sometimes a conspicuous foot. The wall of the capsule has two or more layers—thus differing from the unistratose wall in the Marchantiales. The spore-mother-cells become deeply four-lobed before the first nuclear division. Elaters are always present.

The Jungermanniales have been divided into two series—Anacrogynae and Acrogynae—based primarily upon the position of the archegonium. This division is somewhat artificial as there are some intermediate forms.

In the Anacrogynae the archegonia are borne upon the dorsal surface of the gametophyte and further growth of the shoot is not affected. In the Acrogynae the apical cell of the shoot is finally transformed into an archegonium, and further growth is thus prevented. All of the Acrogynae develop leaves and include the great majority of the liverworts.

ANACROGYNAE

Cavers recognizes four families of Anacrogynae: Codoniaceae, Aneuraceae, Blyttiaceae and Calobryaceae. Goebel (16) unites the second and third of these and part of the first into a single family, Metzgeriaceae, and proposes three additional families—Pelliaceae, Fossombroniaceae and Treubiaceae. This illustrates the very unstable condition of the present classification of these Hepaticae.

About 600 species of the Anacrogynae have been described. As already indicated, the gametophyte may be a quite undifferentiated thallus, *e.g.*, *Pellia*, *Aneura pinguis*, or there may be developed a definite midrib with conducting tissue, *e.g.*, *Pallavicinia*, and various types of leaf-like organs. The development of frond-like branch systems, shown in *Umbraculum* and *Mittenia*, is an interesting case of homoplasy so characteristic of the Anacrogynae.

CALOBRYACEAE (11, 16)

This small family, with only about half a dozen known species, is usually included in the Jungermanniales, but it differs so markedly from any of the other families that it would seem better to establish an order, Calobryales, coordinate with the Jungerman-

niales, Marchantiales and Sphaerocarpaceae. The best known form, *Calobryum Blumei*, has a prostrate rhizome-like stem from which are developed upright shoots bearing three series of conspicuous leaves. The habit is much like that of some acrogynous liverworts, and the apical growth is much the same. However, the structure of the sexual organs and sporophyte differ greatly from those of any of the other Hepaticae and the family seems to be quite unrelated to any other family of liverworts.

ACROGYNAE

The Sphaerocarpaceae, Marchantiales and anacrogynous Jungermanniales are, presumably, old groups of which relatively few forms have survived. Some of the latter, as we have seen, develop leaf-like organs which may closely resemble those of the Acrogynae, and it seems probable that the latter have been derived from some types related to these foliose Anacrogynae. It is highly probable that the existing Acrogynae represent several independent phyla derived from different anacrogynous ancestors.

While there are more than 7000 species of Acrogynae, some seven times as many as all the other Hepaticae, their structure is much more uniform than that of either the Marchantiales or the Anacrogynae. The gametophyte is dorsiventral and has a definite central axis bearing usually three rows of leaves corresponding to the three series of segments formed from the tetrahedral apical cell. Sometimes the ventral row of leaves ("amphigastria") is absent.

The Acrogynae are decidedly the predominant liverwort type, occurring in all parts of the world but reaching their maximum development in tropical mountain forests and moist lowland forests of the southern hemisphere where they form a conspicuous feature of the flora. They may be strictly terrestrial in habit, growing also on rocks or fallen logs, or they may be epiphytes. The epiphytic habit is especially marked in tropical rain forests where they occur not only on the trunks and branches of trees, but many small species grow on the surface of fern fronds and other broad leaves. These "epiphyllous" species are especially abundant in the rain forests.

The epiphytic habit is probably a secondary development and it may be assumed that the terrestrial condition, like that of most

Anacrogynae and Anthocerotae, is more primitive. Some of the acrogynous genera, like *Nardia* and *Lophozia*, much resemble such anacrogynous genera as *Fossombronia* or *Noteroclada*, and it is quite possible that from forms resembling these genera some of the Acrogynae may have been derived. There is great range in size, and the leaves exhibit many peculiarities of form, *e.g.*, the development of special water sacs in *Frullania*, but the tissues show very little specialization and there are no definite conducting tissues. This stereotyped fundamental structure, combined with the great variety of minor variations within it, indicates a highly specialized and presumably relatively modern group, compared with the anacrogynous types.

Just as among the Anacrogynae there occur genera like *Treubia* and *Androcryphia* which suggest the Acrogynae, so among the latter there are genera which, while truly acrogynous, show a marked tendency to develop a thallose condition such as is typical of the Anacrogynae. In these forms the gametophyte at first develops a sort of protonema from which the fertile leafy shoot arises secondarily as a bud. This protonema is sometimes a branched alga-like filament like the protonema of a moss. In other cases it is a flat thallus resembling the simpler Anacrogynae.

The sexual organs do not differ essentially from those of the Anacrogynae. Development of the complete sporophyte is known in only a small number of species, but two markedly different types have been described, and on this basis the Acrogynae have been divided into two "tribes," Jubuleae and Jungermannieae. The differences between the sporophytes in these two groups are such as to suggest that they represent two independent phyla. In the first occur the very large genera *Lejeunea* and *Frullania*, in the other, all the other genera that have, as yet, been investigated (30).

INTERRELATIONSHIPS OF THE HEPATICAE

The Hepaticae apparently represents a natural group whose most specialized members are the Marchantiaceae and the acrogynous Jungermanniales—the latter being the most recent. Assuming that the Hepaticae are all more or less related, the simplest, and presumably most primitive of the existing forms, is represented by *Sphaerocarpus*. From some *Sphaerocarpus* type, the two main lines of development, the Jungermanniales and Marchantiales di-

verged, in the former the differentiation of the gametophyte being mainly external with a marked tendency to form definite leaves. In the Marchantiales the differentiation was internal with formation of a highly developed photosynthetic apparatus, the gametophyte retaining its primitive thallose form.

Assuming that *Sphaerocarpus* is the nearest living representative of the ancestral Hepaticae, it is probable that existing families are the remnants of several independent lines of development that may be traced back to a *Sphaerocarpus* type. In all families of the Jungermanniales there is a tendency to develop definite leaves, this culminating in the modern Acrogynae. The latter probably do not constitute a single closed phylum but represent the end-forms of several independent phyla.

Among the Acrogynae, *Fossombronia* has been suggested as an intermediate form leading up to some of the Acrogynae. On the one hand, *Fossombronia* shows points of similarity to the Sphaerocarpaceae and, on the other, to *Treubia* and *Petalophyllum*, forms with distinct leaves and the tetrahedral apical cell of the Acrogynae. Of the latter group, the Lophoziaceae, e.g., *Nardia* and *Lophozia*, may be distantly related to the *Fossombronia* series. Another independent line that has been proposed is the Lejeuneaceae, some of which in their early stages show a flat thallus like that of some of the simple Anacrogynae, the Aneuraceae. It must be added that these conclusions are not admitted by many students of the Jungermanniales who regard the Acrogynae as monophyletic.

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EXPLANATORY NOTES

By the editors

Acrogynous: growing at the apex.

Archegoniates: all plants possessing an archegonium, a particular kind of female reproductive organ. Includes liverworts, mosses, ferns and gymnosperms.

Elaters: fine hair-like bodies found among the spores of hepatics, consisting of a single cell with walls spirally thickened. They assist in the dispersal of spores by their hygroscopic movements.—Grout.

Gametophyte: the sex cell- or gamete-bearing phase of a plant. It develops from a spore borne on the sporophyte, *i.e.*, by the spore-bearing phase. In Bryophytes the sporophyte is always parasitic on the gametophyte and connected with it by a so-called 'foot' at the base of the 'seta' which bears the 'capsule.'

Hepaticae: liverworts; together with mosses, the Musci, they comprise the Bryophytes, one of the primary divisions of the Archegoniates.

Homoplasny: resemblance, but not involving common origin.

Pyrenoid: a structure connected with chloroplasts of certain green algae and apparently associated with starch formation.

Ulothricales: an order of green algae.

EVOLUTION OF POLLEN GRAINS

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If one examines a random collection of pollen grains, such as might be obtained from a sample of honey (1), the muddy bottom of a pond (10, 14), or from a sticky slide exposed to the winds (23), the forms encountered are surprisingly various, as various, in fact, as the plants which produced them. There may be the one-furrowed form of the magnolia, the smooth single-pored globular grain of grass, or the three-furrowed grains of pea, rose or buckwheat; there may be the minute grains of forget-me-not, so small that they are apt to be entirely overlooked, or the enormous grains of hollyhock and four-o'clocks of several thousand times the bulk; and always there will be the large grains, with their two bladdery wings, of the pines. Some, like those of the grasses, will be smooth, while others, like those of the composites, will be bristling with spines or covered with a reticulum of vertical ridges marking the surface into a geometrical pattern. At first sight, these different forms appear to be entirely unrelated, yet they have all been derived from each other by evolutionary processes quite comparable with those whereby the plants which produced them were derived.

But evolution alone does not give pollen grains their varied forms. In considering pollen grains it is necessary always to bear in mind their rather peculiar mode of formation in tetrads. Nowhere else than among pollen grains are the words of Nägeli (12) truer, when he said: "A correct understanding of a thing can be gained only by a knowledge of its beginning as well as its ending." A correct understanding of pollen grains can be gained only by considering them against a background of their generation. The fact that they originate in tetrads is almost invariably the primary moulding force which gives them their completed form.

Pollen grains are formed from a pollen mother-cell in a way which is almost unique in cell formation. The nucleus nearly always goes through two divisions in rapid succession, followed by two *successive* or *simultaneous* divisions of the cell, forming four

new cells which, by growth and further development, become pollen grains. At maturity, however, they are generally quite separate and it is difficult to see just what the effects of their contacts with each other were. In some few cases, however, the grains never separate, but remain united in tetrads throughout their life; in many others, occasional monstrosities occur, the grains remaining united at maturity in the same relative positions in which they were formed, a sort of "Siamese" quadruplet. We learn from a study of these that pollen grains may be associated with each other in all possible arrangements of four cells in contact, but the two arrangements most commonly observed are: (1) the tetrahedral, in which the grains lie in a compact cluster, so-called because they occupy the relative positions of the angles of a tetrahedron; (2) the tetragonal or flat arrangement, in which they occupy the positions of the four angles of a square or rhombus. The former is a least-surface configuration and results from a postponement of the first division of the pollen mother-cell until after the second division of the nuclei, enabling the four daughter nuclei to slip into the least-surface, or most compact, configuration before the cell walls are formed. The second is the result of two successive divisions—generally bipartition with rectangular intersection, which compels the cells to occupy the positions relative to each other assigned to their nuclei at the time of their division. The simultaneous division (Fig. 1) of the tetrad with its resultant tetrahedral arrangement is prevalent among higher dicotyledons (4, 7), and the successive division is prevalent among primitive dicotyledons, monocotyledons and gymnosperms. But there are so many exceptions that this can scarcely be called a rule. Nevertheless, the way in which the grains are formed determines, in large measure, their ultimate shape.

There are three basic forms of pollen grains or microspores from which, it appears, all others are derived. *First*: There is the form with the triradiate crest, marking the boundaries of the three faces of contact the grain made with its neighbors of the tetrad (Fig. 2A). Each of the radii of the crest is a double ridge which may easily split open, providing lines of dehiscence through which the sporoplast emerges at time of germination. This form is common among the Filicales and primitive gymnosperms, but among angiosperms is known to occur only in the Magnoliaceae. *Secondly*:

There is the one-furrowed or monocolpate form (Fig. 2B). It may be elongate or rounded but is always provided with a single germinal furrow which forms on the side of the grain remote from contact in the tetrad. This furrow serves as a place of emergence for the pollen tube at time of germination. This form characterizes the grains of most gymnosperms, monocotyledons and lower dicotyledons. *Thirdly*: There is the three-furrowed or tricolpate form, provided with three germ pores or three meridionally arranged germinal furrows (Fig. 2C). Each pore or furrow forms at a point of contact that the grain makes with its three neighbors of the tetrad, and one or more of them serves as a place of exit for the pollen tubes. This type of grain characterizes all higher dicotyledons, though it is more often than not profoundly modified, so that it is not easily recognizable. These three basic forms represent three different modes of response to contact stimuli received in the tetrad. In the first, the stimulus results in the flattening of each of the faces of contact with the formation of ridges between them; in the second, no visible effect is produced at sites of contact, but their stimulus forces the formation of the pore or furrow to the opposite side of the grain; in the third, the stimulus induces the formation of a pore or furrow on each of the contact faces. These different modes of response denote huge genetic differences, yet they are not entirely unrelated.

If we examine the most primitive form of pollen grain we find it to be a simple, rounded, protoplasmic body enclosed by a coat in which an inner and outer layer can be distinguished, the outer firm and protective, the inner delicate but impervious; the two layers are thus comparable to the casing and inner tube of an automobile tire. This description would suit a fern spore equally well, which the most primitive pollen grains very closely resembled. Grains of this kind are found among fossil Cycadofilicales, which were not greatly in advance of the ferns. For example, a pollen grain of the Paleozoic *Crossotheca* found by Kidston (9) is virtually the same as a fern spore. It has a triradiate crest on one side, marking off the three faces which were in contact with its three neighbors of the tetrad (Fig. 2A). Its opposite side, which was outward in the tetrad and which for convenience we will call the ventral, was rounded. Such a grain had no germ pore or germinal furrow. Germination here was similar to that of a fern spore; the nucleus

went through several divisions forming a prothallus which, as it grew, ruptured the cell wall, apparently through the triradiate crest and threw it off. We see a similar development in the grain of the fossil *Aetheotesta elliptica* (Fig. 4 *Cycadofilicales*) which was found in its pollen chamber by Renault (15), divested of its outer coat. Then, it is believed, the cells of the prothallus formed motile spermatozoids which were set free in the pollen chamber and brought about fertilization.

This simple pollen grain had no performed germ pore or furrow, but like all other pollen grains and fern spores too, its contents must have had the property of readily taking up and giving off water with consequent increase and decrease in volume, and since this could not be accommodated by any change in shape of the dorsal surface, on account of the stiffening effect of the triradiate crest, changes of volume must have been accommodated by the ventral side of the grain. This we may readily see in such a fern spore as that of *Osmunda* (Fig. 3). When the *Osmunda* spore dries, its contents shrink and a large and deep concavity forms on the ventral surface and, though the spore itself was originally spherical, the concavity is generally elongate. Here, then, is the most primitive germinal furrow, merely a tucking-in of the ventral surface, its position forced there by the presence on the dorsal surface of the triradiate crest or, if the grains of the tetrad failed to separate, by the presence of its three neighbors.

It will be noticed in the foregoing example that the ventral furrow primitively served as a mechanism to accommodate changes in volume and not as a place of exit for a pollen tube; the germination of such fern spores as that of *Osmunda* takes place by dehiscence through the triradiate crest. Moreover, this function of the furrow of accommodating changes in volume has been retained, together with its more recently acquired function of permitting the exit of the pollen tube, among the higher gymnosperms and angiosperms. For this reason I have coined for it the term *harmomegathus* which means an organ of volume-change accommodation.

The next stage in the evolution of pollen grains is perhaps represented by those of *Dolerophyllum* (15, 16) and *Whittleseya* (19), among the Cordaitales (Fig. 4). These are fossil trees which lived in the Mesozoic period and were probably the precursors of our modern conifers. Their pollen grains differed from those of the

Cycadofilicales in having a well defined preformed furrow on the ventral side. It appears that, as the prothallus of these grains developed upon reaching the pollen chamber of the seed they were to fertilize, the invaginated furrow became evaginated and finally separated from the rest of the exine, opening like a lid, and permitted the escape, into the pollen chamber, of motile spermatozooids. Here, then, was a preformed organ which was both a harmomegathus and a germinal furrow; it permitted changes in volume due to changes in moisture; it permitted the growth of the prothallus without prematurely rupturing the spore coats; finally, it split off, and permitted the escape of the fertilizing elements. But in these grains the prothallial tissue was much less extensive than in those of the Cycadofilicales. The reduction of prothallial tissue which marks the evolution of pollen grains all the way up to the angiosperms had already set in.

The next stage in the evolution of pollen grains is perhaps represented by the Bennettitales (Fig. 4). Here prothallial elimination has proceeded much further. Occasionally, the whole grain was partitioned into a few large cells, but more often only a few rounded cells were cut off and pressed against the inside of the pollen wall. With this prothallial reduction, however, there is not found a corresponding reduction in the size of the furrow; it still persists as a wide-open gash reaching from end to end of the grain, yet much less necessary than before (2, 21, 22).

A further step forward is seen in the grains of *Ginkgo* and the Cycadales. Here is found virtually complete prothallial elimination. At germination, a sort of pollen tube is formed; it functions somewhat differently, however, from that of the grains of angiosperms. But the germinal furrow still persists, extending the whole length of the grain (Fig. 4). It has no means of closing on account of its rounded ends and it appears to be enormously larger than necessary, leaving the grain wide open over a large proportion of its ventral surface (8, 17). It may be a significant fact that the four great groups, Cycadales, Bennettitales, Ginkgoales, and Cordaitales, which possessed grains with the wide open furrow, are now either nearly or quite extinct.

This type of grain, since it was common to all the more primitive forms, must have been the natural heritage of higher gymnosperms, such as the conifers, and of angiosperms, whatever their

origins may have been. And as we pass in review the grains of the different groups, I think there can remain no doubt that the effects of this wide-open furrow, which had been developed to meet a need which no longer existed, were, in its new associations, more detrimental than beneficial. The evolution of pollen grains from this stage onward is largely the story of the modification, protection, reduction or elimination of the wide open furrow which was their heritage from the past. The ways in which the different groups disposed of it forms one of the most dramatic chapters in the whole of pollen morphology.

Among conifers we find a number of rather obvious ways in which this was done. Curiously, each way characterizes, for the most part, one or two of the tribes and suggests that each may represent a separate line of development. The Araucarineae, regarded by many as the most primitive of gymnosperms, exhibit, perhaps, the simplest way. The floor of the furrow appears to have been evaginated and its exine thickened. In some, for example, *Agathis* (Fig. 4), the grains are spherical with exine of uniform thickness throughout so that one could not guess that they bore any relation to one-furrowed grains, but in others, for example, *Araucaria* (Fig. 4), a rim-like thickening is quite evident, which can only represent the rim of the all-but-vanished furrow.

The Podocarpineae, also primitive and in some ways similar to the Araucarineae, disposed of the furrow by developing a wing-like bladder around it. This is so arranged that when the grain dries and contracts the furrow dips in, causing the frill to buckle together, tightly closing the gap. The ability to develop this bladder-like frill was undoubtedly inherited from the remote past. Similar bladders are found on grains of some ancestral Cycadofilicales as, for example, *Stephanospermum caryoides* (Fig. 5). In this case, the frill was developed probably as a floating organ for its possessor lacked a germinal furrow; it was a large grain, however, about a hundred microns in diameter, so that some floating device was necessary to enable it to reach its goal. Among podocarps, however, floatation does not seem to be the primary function of the bladder-like wings for the grains are small enough to float unaided; in fact, they are no larger than those of most wind-pollinated angiosperms which manage quite well without any floating devices. Among podocarps, the bladder-like frill became an organ of

protection for the furrow. In response to this new function it became modified in two distinct ways. When the furrow was elongate the frill became divided into two halves which close over the furrow like the shells of a clam (Fig. 4, *Podocarpus*). But if the furrow was not elongate the frill became separated into three detached bladders which served the same function (Fig. 4, *Pherosphaera*). It should be noticed, however, that the grains of *Saxegothaea*, which is generally regarded as belonging to this group, are without bladders, resembling, in this respect, the grains of the Araucarineae (20).

Pollen grains with similar bladders are found among the Abietineae, for example, those of *Pinus*, *Abies*, *Picea*, *Cedrus* and *Pseudolarix* (Fig. 4); but it is not a universal character in this tribe either, for bladders are entirely lacking in the grains of *Tsuga*, *Larix* and *Pseudotsuga* (Fig. 4). The presence of bladders on the grains of the Podocarpaceae and Abietineae has suggested to many investigators that the two tribes may be related. In other respects, however, the grains of these two groups are quite different; among podocarps, in those grains which have two bladders the furrow is always sharply defined with a distinct rim to which the ventral roots of the bladders are attached, whereas among the winged grains of the Abietineae the furrow is not sharply defined and has no rim. Furthermore, the grains of the Abietineae are generally about three times as large in their linear dimensions as those of the Podocarpaceae. The possession of bladders seems to be merely one of those characters which appear again and again throughout large groups and immense periods of time; it is even older than the furrow itself.

Another method of dealing with the furrow was by reducing its size. In the grains of *Taxodium* and *Torreya* (Fig. 4) we find it represented by a small and slightly elongate weak spot on the ventral side, which bulges somewhat when moistened but seems too small and ineffective to be of any importance in accommodating changes in volume. In the grains of *Cryptomeria*, *Sequoia* and *Glyptostrobus* (Fig. 4) of the Taxodineae, the furrow is pinched up into a pointed papilla which takes no part whatever in accommodating the grain to changes in volume; when these grains dry and contract the whole ventral surface dips in, saucer-like, with the papilla standing up in the middle. Grains of *Glyptostrobus* are

often united in their tetrads and the little papillae always face outward, showing by their position that they are really homologues of the germinal furrow, though they are so far reduced that they might not otherwise be recognized as such.

A further step in the reduction of the pore is found in the grains of *Cunninghamia* (Fig. 4). Here the papilla is only a vestige, so small and insignificant that it cannot always be seen, if, indeed, it is always present. Total elimination of the furrow is found in the grains of *Taxus*, and in those of *Juniperus* and *Thuja* (Fig. 4); in fact, throughout the Cupressineae.

The question naturally arises: What takes the place in these grains of the vanishing furrow? It had two very necessary functions, germinal emergence and volume-change accommodation, which cannot be dispensed with. As the furrow was progressively reduced, first to a pore, then to a papilla, and then eliminated, there was a progressive reduction in thickness of the exine and increase in thickness of the intine. In the grains of *Juniperus*, which we may take as the culmination of this line of development, the exine is thin and flexible, easily accommodating ordinary changes in volume, and there is an enormously thickened intine which, upon germination, swells, ruptures the exine and throws it off completely, the grain developing thereafter as a naked prothallus—a curious reversion to the ancestral method of the Cycadofilicales.

Still another way of dealing with the furrow was adopted by the grains of *Welwitschia* and *Ephedra* (Fig. 4) which are probably best regarded as advanced gymnosperms. In *Welwitschia* the grains retain their thick exine. The furrow is simply floored over by the thick and inelastic material of the exine, thereby greatly impairing its function of harmomegathy. This function is taken over by a large number of grooves and ridges which enable the grain to change its size and shape without rupturing its walls. The furrow, though quite evident, plays but little part in the adjustment. In the grains of *Ephedra*, which is undoubtedly related to *Welwitschia*, the process of furrow reduction is carried a step further. In those of most species the furrow is entirely absent, its function being taken over by the grooves and ridges with which these grains are still better provided than those of *Welwitschia*; either these are small and numerous, as in the grains of *Ephedra altissima*, or they are large and fewer in number and of a highly specialized character

as, for example, those of *Ephedra glauca* (Fig. 4). The exine of these grains is very thick and inelastic, permitting no stretching, but this is compensated in the grains of *E. glauca* by a thin zigzag streak of elastic material in the bottoms of the grooves, giving off branches outwards toward the crests of the ridges. These permit the grain readily to change its shape by allowing the ridges to become more flattened and broader and at the same time more arched throughout their length as the grain expands, and at time of germination afford lines of dehiscence. In its extraordinarily specialized grain, *Ephedra* seems to stand very high among gymnosperms or represents a highly developed group coordinate with them.

Among gymnosperms we have thus seen a number of different ways of dealing with the wide open furrow which was their heritage from the past. In the Araucarineae it was pushed out and floored over, among the Podocarpaceae and Abietineae lateral bladders were developed which fold over it, in the Taxineae it was reduced to a pore, in the Taxodineae to a papilla, and in the Cupressineae completely eliminated. It was simply floored over with its impaired harmomegathic function transferred partly to a large number of longitudinal grooves in *Welwitschia*, and completely eliminated and its functions entirely taken over by longitudinal grooves in the grains of *Ephedra*. As far as evidence of the pollen grains can tell us, these various methods of disposing of the furrow may represent as many lines of development, that is, coordinate branches of the gymnosperm stock. But it does not imply that the Podocarpaceae are closely related to the Abietineae because both tribes developed bladders in some of their genera, nor that the Taxineae are related to the Taxodineae because in both of them the furrow is reduced to a papilla. These could as well be duplications of the same method of disposing of the furrow in coordinate groups.

The same primitive single-furrowed type of grain appears also to have given rise, perhaps directly, to those of most monocotyledons. It is true that there is much variation among the grains of this group but, outside of the Alismataceae and a few associated groups which appear to be related to the Ranales, there is nearly always just one furrow or pore or none at all, for all through the group, as among gymnosperms, there is a strong tendency to do away with

the furrow. The grains of the Palmaceae (Fig. 4) are one-furrowed, almost the same as those of primitive gymnosperms; their only advance over those of cycads is an increase in length and the more pointed ends of the furrow, which permit the latter to close tightly. The grains of the Liliaceae and allied families, of Typhaceae, Sparganiaceae, Juncaceae and others, though often highly modified, are likewise clearly derivatives of the one-furrowed type. In the grains of the Musaceae and Cannaceae (Fig. 4, *Canna*) the furrow is completely eliminated with the exine extremely thin and the intine extremely thick, comparable, in this respect, to the grains of *Juniperus*. In the pollen grains of the grasses (Fig. 4, Gramineae) the furrow is reduced to the smallest possible pore, provided with a little lid or operculum which closes it tightly when the grain dries.

The Nymphaeaceae are somewhat anomalous in their position; in some characters they are monocotyledonous, in others dicotyledonous. It is, therefore, interesting to see what their pollen grains tell about them. The pollen grain of *Castalia* (Fig. 4, Nymphaeaceae) is shaped something like a turtle bereft of its appendages, with an upper and lower shell and between them a ring-shaped strip of elastic membrane. When this grain dries the flattened side is drawn inward and when it is moistened it is pushed out again, its free movement being permitted by the thin elastic membrane which surrounds it. The grain of *Nymphaea*, the yellow water-lily, is similar, except that the furrow is elongate and its enclosed area of the exine is a narrow strip which becomes completely tucked in when the grain dries. At first sight one would be tempted to say that these grains have a ring-shaped furrow, entirely different from anything we have yet seen. But if we examine them a little closer we find that the rounded or dorsal surface is generally covered with long spines, while the exine of the ventral surface, which is surrounded by the ring of thin exine, is generally nearly or quite smooth; furthermore, there is often a distinct difference in the texture of the two surfaces showing that the area enclosed by the ring is not just a part of the exine cut off from the rest. A more correct interpretation seems to be that the grain has one large furrow occupying the greater part of its ventral surface, and the detached piece of exine is its operculum, morphologically its thickened furrow floor.

Interpreted in this way, we see in the Nymphaeaceae just another way of dealing with the furrow. The pollen of the Nymphaeaceae, therefore, suggests that the plants may not be either monocotyledons or dicotyledons, but should perhaps be regarded as a coordinate group, possibly derived from the Bennettitales and on a par with the Magnoliaceae which are regarded by some as the direct descendants of the Bennettitales.

The one-furrowed type of grain, while it is characteristic of the gymnosperms and monocotyledons, is not confined to these groups. It reaches a little way up into the dicotyledons in surprising fashion (5, 6). It is found without any important modification in the Saururaceae, Piperaceae (Fig. 4, *Piper*) and Chloranthaceae, which are regarded as among the most primitive members of the dicotyledons, and the possession of this type of grain by these groups is a most remarkable confirmation of the position assigned to them at the beginning of the dicotyledonous series. Outside of these admittedly primitive families, the one-furrowed type of grain is found also in the Magnoliaceae (Fig. 4, *Magnolia*), which is in keeping with the position assigned to them in most modern classifications. But it is not found in the Salicaceae, Juglandaceae, Betulaceae, Casaurinaceae, and other Amentiferae (24) which are also sometimes regarded as primitive. The grains of these families show unmistakable signs of reduction and apparently trace their origin to some of the higher dicotyledons.

The pollen grains of the remaining dicotyledons are entirely different from those of the lower dicotyledons and gymnosperms (24). Instead of having a single furrow or pore forming on the part of the grain most remote from contacts with its neighbors of the tetrad, the basic form of grain of the higher dicotyledons has three pores or furrows which form at the points of contact that the grain makes with its neighbors of the tetrad (Fig. 2C). The organizations of the two types of grain are thus fundamentally different and, for the most part, must represent an enormous genetic gap, but, by the most extraordinary good fortune, we find that gap nicely bridged in the Magnoliaceae.

The Magnoliaceae are generally regarded as consisting of three tribes, Magnolieae, Illiceae and Schizandreae. There is a tendency nowadays, though, to regard the two latter tribes as belonging to a separate family, the Schizandraceae. Judging by their pollen

forms, there still seems to be some relationship between the two families, distant perhaps but significant. Therefore, in this discussion I prefer to retain the older classification. The pollen grains of the tribe Magnolieae are one-furrowed and scarcely to be distinguished from those of the Bennettitales. Wieland (21) says: "The Magnoliaceae must be among, if not the most primitive of all the angiosperms." He regards such species as those of *Magnolia* and *Liriodendron* as directly descended from the Bennettitales—little more than modernized williamsonias or wielandias. The pollen grains of the tribe Magnolieae, in their simple and unmodified single furrow, suggest that they are certainly among the lowest angiosperms, perhaps on a par with the Saururaceae and Piperaceae though not necessarily closely related to them, because their grains are very much larger.

Somewhat similar are the pollen grains of *Drimys* (Fig. 6) of the tribe Illiceae, but their particular interest lies in the fact that they remain united in their tetrads at maturity. Since the tetrads are tetrahedral the grains are rounded or somewhat triangular, and each has a single furrow facing outward, and which is rounded instead of elongate, but it functions in the same way as in the grains of the Magnolieae, bulging outward when moistened and dipping inward when dried, and serving as a place of emergence for the pollen tube at time of germination. The main points of interest in this grain lie in the fact that it shows plainly that the position of the single furrow is on the distal side of the grain in the Magnoliaceae in relation to its tetrad, as it is in grains of primitive gymnosperms. That this relation is practically universal among pollen grains was first observed by Fischer (5). I have, therefore, designated it as Fischer's law.

The grain of *Illicium floridanum* (Fig. 7), which belongs to the same tribe as *Drimys*, is quite different. It does not have any trace of the large single furrow. Instead, it has three slender furrows which reach from pole to pole, dividing the exine of the grain into three equal lunes. These three furrows appear not to be homologous with the three furrows of the ordinary dicotyledonous grain. They do not participate in harmomegathy—these grains are thin-walled and shrink by simple collapse in one or more of their lunes—and they do not provide places of emergence for the pollen tubes. Instead, they provide lines of dehiscence along which the exine

splits completely apart into three separate sections which are cast off. In this respect they resemble the triradiate crest of the fern spores with which, it therefore appears, they are homologous, though this point has not yet been settled. If this interpretation is correct we have here a curious survival of an extremely ancient type; it is the only example of dehiscence that I know among pollen grains of angiosperms. It seems a curious paradox that of the grains of *Drimys* and *Illicium*, which are conceded to be closely related, one should possess an ancient pteridosperm feature and the other a gymnosperm feature; the significance of this paradox, however, becomes more apparent when we consider the grains of the next tribe of the family.

In the grains of *Schizandra* (Fig. 8), of the tribe Schizandreae, there are six furrows meridionally arranged. Three of these are long and meet at one pole but end blindly about 45 deg. short of the opposite pole, and three of them are short and do not meet at either pole. These latter alternate with the long furrows crossing the equator by which they are nearly or quite bisected. At the free pole, where no furrows meet, the exine is flexible and may dip in or bulge out in response to changes in volume. The three furrows which meet at the opposite pole provide lines of dehiscence through which the pollen protoplast may emerge; they are the homologues of the triradiate crest of the fern spores and pteridosperm spores. It is, therefore, safe to assume that the pole at which they meet was proximal in the tetrad and that they represent the boundaries of the three faces of contact that the grain made with its neighbors of the tetrad. The three short furrows appear to serve no function, unless they merely stiffen the exine and so limit the flexible area to the region of the distal pole. Nevertheless, their position across the contact faces between the long furrows shows that they are morphologically homologous with the three furrows of the higher dicotyledons. The flexible area around the distal pole corresponds to the single furrow in the grains of gymnosperms. Thus we find combined in this remarkable grain the main features of the pteridosperms, of the higher gymnosperms and of the higher dicotyledons, the three basic types which represent three different responses to contact stimuli in the tetrad.

It is true that the three short furrows of the *Schizandra* pollen grain accommodate neither changes in volume nor pollen-tube emer-

gence as their homologues do in the pollen grains of higher dicotyledons, the former function being provided for by the flexible polar area and the latter by dehiscence through the point of confluence of the three long furrows. Nevertheless, the structure of the three short furrows is exactly the same as that of the long furrows, so it seems reasonable to suppose that, among higher dicotyledons, with loss of the triradiate crest which is entirely absent from their grains, these three short furrows could easily have taken over their function by simply splitting longitudinally and, having once acquired a longitudinal split, they could likewise take over the function of volume-change accommodation. Such appears to be the origin of the three furrows which characterize the pollen grains of higher dicotyledons. In grains of *Schizandra* they function only as structural stiffening of the walls. In grains of higher dicotyledons they represent the taking-over of two additional functions coincidentally with elimination of the organs which formerly performed them.

Furrows of this type belong strictly to pollen grains of higher dicotyledons; they are not found elsewhere. Development of these furrows was the great achievement of the dicotyledonous pollen grain. With it the grain was released from limitations imposed upon it by the single long deep furrow which had been its heritage from ancestral gymnosperms of the remotest antiquity. With this release came the most astonishing diversity of form developed through the relatively short succeeding span in the evolutionary scale, standing in remarkable contrast to the continuous monotony of the preceding development of the one-furrowed grain. Through some such form as that of *Schizandra* the grain appears to have been set free with the acquisition of a new set of organs allowing it a new way of doing things, and of this the enormous variety of pollen forms among higher dicotyledons is the expression.

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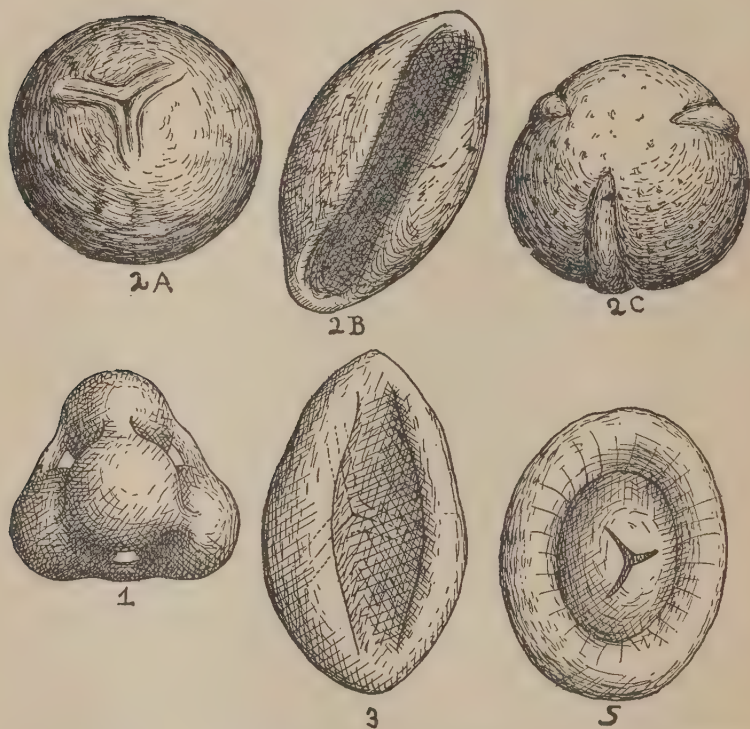


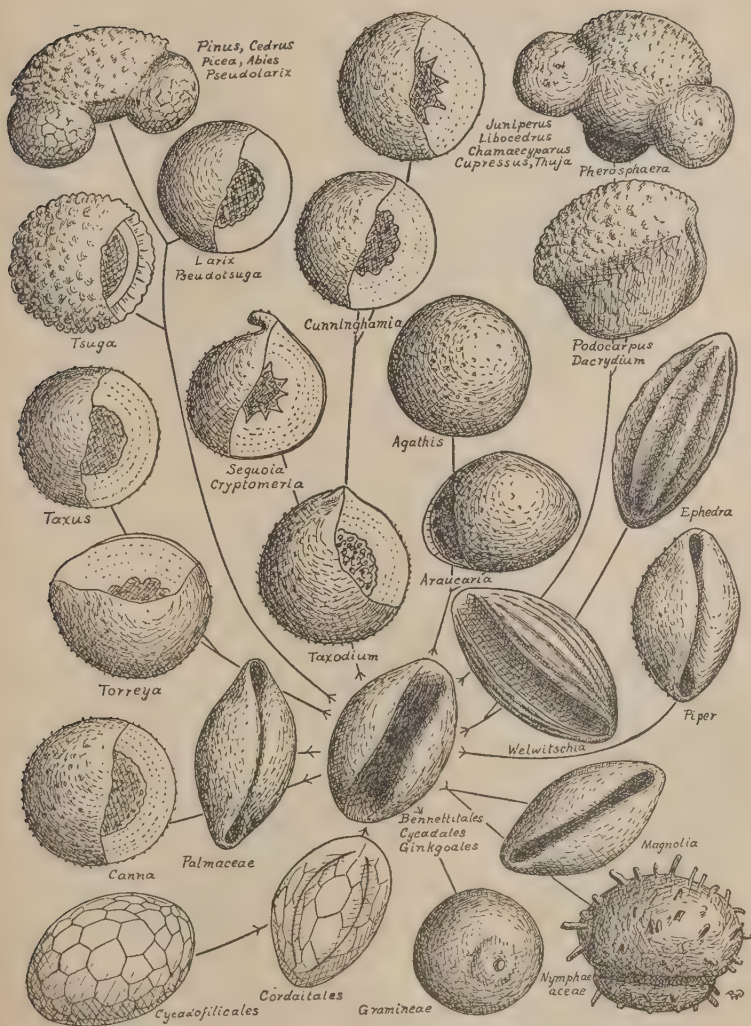
FIG. 1. Pollen tetrad in the tetrahedral arrangement with the four daughter cells about to separate, diagrammatic.

FIG. 2. Three basic forms of microspores and pollen grains; A. with triradiate crest; B. with single furrow on the ventral side, monocolpate; C. with three furrows meridionally arranged, tricolpate.

FIG. 3. *Osmunda* spore, diagrammatic, ventral view with its single temporary furrow uppermost, drawn as if transparent to show the triradiate crest on the dorsal side.

FIG. 4. Representative pollen grains, semi-diagrammatic, showing the sequence in which the various forms might have been derived from each other. Reproduced, with modification, from *Pollen Grains* by the present author (24), with permission of the McGraw-Hill Co., Inc., New York.

FIG. 5. Pollen grain of *Stephanospermum caryoides*. After Oliver (13).



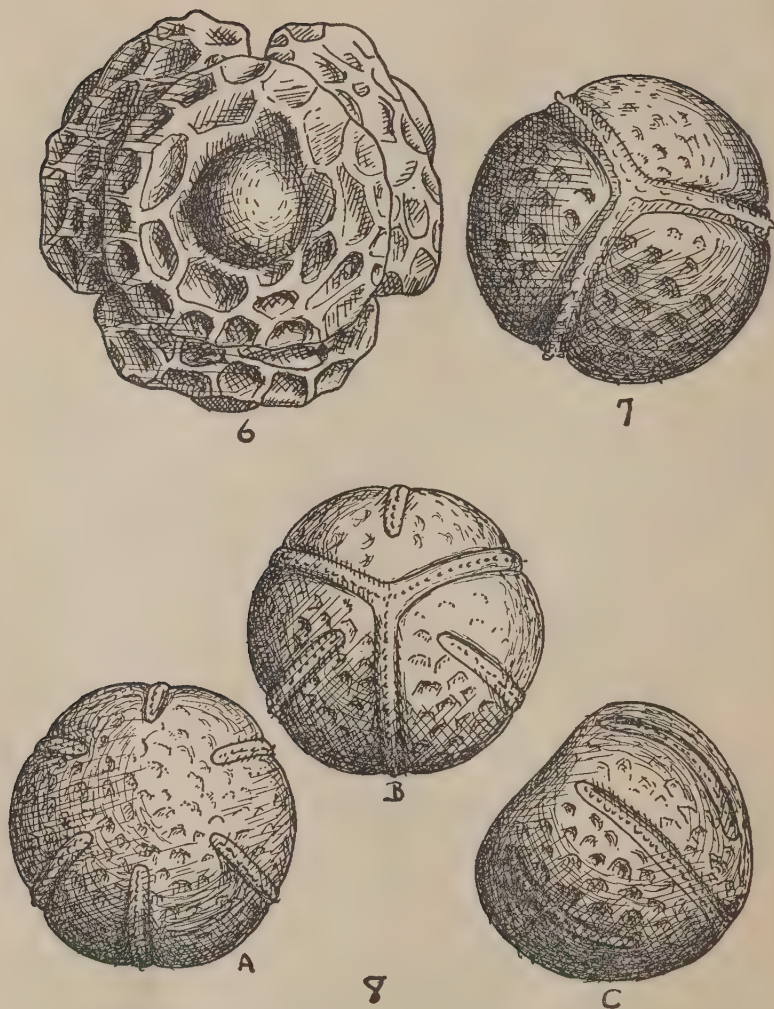


FIG. 6. Tetraglobate pollen of *Drimys Winteri*, 47μ in diameter.

FIG. 7. Pollen grain of *Illicium floridanum*, polar view, 28μ in diameter.

FIG. 8. Pollen grain of *Schizandra chinensis*, 21.6μ in diameter, three views, A. ventral, B. dorsal, C. side.

CYTOPLASMIC INCLUSIONS OF PHYTOMASTIGODA*

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INTRODUCTION

At the present time, there is a certain amount of disagreement as to the nature and identity of the various types of cytoplasmic inclusions in the Phytomastigoda. According to one viewpoint (9), there should be recognized a *vacuome*, consisting of definite vacuoles or smaller inclusions characterized by certain staining reactions, and an *ergastome* composed of "liposomes"; the term *chondriome* is applied only to the so-called initial stages in the development of the vacuome. In another interpretation (20), which is in better accord with the views of the majority of cytologists, the vacuome and the *chondriome* are considered to be entirely distinct types of cytoplasmic inclusions, differing in physico-chemical nature and in staining reactions. As for the identity of the Golgi material in the plant-like flagellates, a number of different views have been expressed. In addition to these more fundamental differences of opinion, the literature contains conflicting reports concerning the identity of the various types of inclusions in specific flagellates.

MITOCHONDRIA (CHONDRIOME)

The mitochondria of the Phytomastigoda have in most instances been identified on the basis of their reaction to Janus green B in vitally stained preparations, and similar inclusions have been observed in permanent preparations; in a few instances, however, the mitochondria have been identified only in fixed and stained material.

Among the Chrysomonadida, small, relatively numerous granules, stainable vitally with Janus green, have been described as mitochondria in *Chromulina* sp. (23) and similar small inclusions have been stained with hematoxylin after osmic fixation. In *Chilomonas paramecium* (Cryptomonadida) the mitochondria have been reported (23) as granular inclusions similar to those of *Chromu-*

* The Phytomastigoda constitute a group of flagellate infusorians which possess chlorophyll bodies, plant-like nutrition and a deficient mouth. It is suggested that Sharp's "Introduction to Cytology" be consulted for cytological terms in this article.—Editor.

lina. In *Noctiluca scintillans* (Dinoflagellida) both bacilliform and granular mitochondria have been described (6), the former being found in the cytoplasm immediately surrounding food vacuoles and the more numerous spherical mitochondria distributed throughout the cytoplasm.

In *Euglena gracilis* spherical mitochondria, relatively few in number, have been reported (5) in permanent preparations. Larger spherical bodies, identified as pyrenoids, are regarded by Causey as being derived probably from mitochondria. In another investigation (24) both rod-like and granular inclusions have been described as mitochondria in the same species. Brown (3)) found only spherical mitochondria in permanent preparations of *E. gracilis*, and Baker (1) likewise reported numerous granular mitochondria scattered throughout the cell. In *Colacium vesiculosum* (28) small rod-like bodies, mostly peripheral in distribution, are demonstrable with Janus green and have been stained with hematoxylin after fixation in Champy's fluid. In *Astasia* sp. (23) numerous small granules, scattered through the cytoplasm, have been stained vitally with Janus green and demonstrated with hematoxylin in permanent preparations after osmic fixatives. *Menoidium incurvum* (24) shows both granular and bacilliform inclusions when stained vitally with Janus green. In *Entosiphon sulcatum* (30) the mitochondria are relatively small rods, peripherally located and staining faintly with Janus green and also with hematoxylin after fixation by Benda's method. In *Peranema trichophorum* (22) numerous elongated subcuticular inclusions arranged in spiral rows are demonstrable vitally with Janus green and by suitable methods of fixation and staining. The mitochondria of the same species have also been identified (4), in fixed and stained material, as inclusions ranging from small spheres to large discoid structures, whereas Grassé and Poisson (18) have described granular mitochondria in specimens stained vitally with Janus green.

In the Euglenida, in particular, it seems probable that the so-called 'mucus-bodies' described by certain workers have occasionally been identified as mitochondria by some investigators, as well as "une simple modification du vacuome ordinaire" by others (8). Thus, inclusions similar in distribution to the peripheral "mitochondria" of *Peranema* (22) and *Colacium* (28), have been described as mucus-bodies in *Euglena granulata*, *E. proxima* and *E.*

viridis (12), and in *E. intermedia* (18). These inclusions are more or less rod-like in *E. proxima* and *E. intermedia*, but are small and granular in *E. viridis*. The mucus-bodies are said (18) to be stainable vitally with neutral red, and are reported (12) as showing an acid reaction in comparison with the more nearly alkaline reaction of the vacuome. In preparations of *Euglena* stained with mixtures of neutral red and Janus green, the mucus-bodies as well as the vacuome are found (18) to be stained with neutral red. In *Peranema* (22), on the other hand, the comparable subcuticular inclusions were stained by Janus green and not neutral red in similar preparations, while the elements of the vacuome were stained with the latter dye. These mucus-globules are said to contribute to the formation of the gelatinous membrane characteristic of the resting stages of Euglenidae. In *Euglena intermedia* (18) each mucus-body is said to lie in a small sac which opens through a fine canal to the outer surface of the organism. In addition to the cases recorded in Euglenida, an 'appareil mucifere' has been described in *Oxyrrhis marina* (10).

In *Polytoma uvella* (Phytomonadida) mitochondria in the form of short rods, and occasionally granules, have been reported (34) as being scattered through the cytoplasm. In *Chlamydomonas* sp. (25) the mitochondria are evident as granules and rods, most of them apparently lying near the surface of the cell. In *Haematococcus pluvialis* (14) numerous small inclusions, usually bacilli-form and mostly peripheral in location, are stained vitally with Janus green.

VACUOME

The vacuome of the Phytomastigoda consists of vacuoles or smaller globules or granules stainable vitally with neutral red, brilliant cresyl blue, and certain other dyes, and may be distinguished from the chondriome by staining with a mixture of neutral red and Janus green. It has been pointed out (8) that there is a striking contrast between the vacuome of the great majority of plants and that of the lower organisms. In the latter the vacuome is usually in the form of small globules or granules rather than vacuoles in the strict sense, whereas the reverse is true of the higher plants. These smaller elements of the vacuome have been designated variously as chromatic granules, metachromatic

granules, volutin granules, fuchsinophile granules, and also as 'chromidies' (8). In sealed-slide preparations the vacuome may often be blackened with osmic acid under direct observation after being stained previously with neutral red (22, 23, 24, 25), the process of impregnation requiring several days. Furthermore, the vacuome is consistently impregnated by the usual osmic and silver Golgi methods without previous staining with vital dyes. In some species it has been possible to observe similar inclusions in the living unstained organism, and in neutral-red preparations to follow the gradual staining of these same inclusions. On the basis of present evidence, therefore, it seems that in the Phytomastigoda, as in plants in general (21), the vacuome consists of normally pre-formed inclusions which may be stained vitally with neutral red and other vital dyes and may be impregnated by the osmic and silver Golgi techniques.

In *Chromulina* sp. (23) a number of small globules are stainable vitally with neutral red, brilliant cresyl blue and neutral violet. After vital staining with neutral red, the globules may be impregnated with osmic acid under direct observation. Similar inclusions are impregnated in the Mann-Kopsch (Weigl) osmic technique. In *Chromulina maxima* (10) the vacuome consists of relatively few globules, which may be almost completely absent in some specimens. In this case the volume of the vacuome is reduced, as compared with that in many other species of flagellates. In *Synura uvella* (10) the vacuome is stained with extreme difficulty with brilliant cresyl blue, and is usually not stained with neutral red. In *Chilomonas paramecium* (23) the vacuome is represented by globules somewhat larger and less numerous than the mitochondria, but similar to the latter in distribution. The vacuome of *Oxyrrhis marina* (10) consists of scattered globules, said to contain 'metachromatin.'

In *Euglena viridis* numerous small scattered granules have been identified both as vacuome (8) and as mucus-bodies (12). A similar situation exists in regard to *Euglena velata*, in which rod-like inclusions have been identified as "aspect en bâtonnet du vacuome" (8) and as 'mucus-bodies' (12). Grassé and Poisson (18), however, have pointed out that the vacuome and the 'mucus-bodies' are two distinct types of inclusions. The vacuome of *Euglena proxima* (15) consists of small scattered globules, stain-

able vitally with neutral red and reacting to osmic impregnation. Similar inclusions have been described (24) as the vacuome in *Euglena gracilis*. The 'pseudochondriome' recognized by Brown (4) in this species may possibly correspond to the vacuome reported by other workers. According to Baker (1) the vacuome of *E. gracilis* shows a "light orange reaction to intra-vital neutral red." Similar orange granules were observed by Hall (24) in unstained specimens and were interpreted as cytoplasmic pigment granules. It seems possible, therefore, that Baker may have mistaken the orange pigment granules for elements of the vacuome in his preparations. The vacuome of *Colacium vesiculosum* (28) consists of small globules scattered through the cytoplasm, and in *Astasia* sp. (23) similar inclusions are stainable vitally with neutral red, brilliant cresyl blue and neutral violet. Globular inclusions of the same type have been identified (24) as the vacuome in *Menoidium incurvum*. In *Peranema trichophorum* (22) the vacuome is represented by numerous small globules scattered through the cytoplasm.

The vacuome of *Polytoma uvella* (34) is composed of metachromatic granules stainable vitally with neutral red, brilliant cresyl blue and Nile blue. The inclusions are demonstrable also by methods for staining metachromatic granules and they are impregnated by the usual osmic and silver Golgi methods. In *Chlamydomonas variabilis* (8) the vacuome may be represented either by numerous small globules ('chromidies') or by larger 'vacuoles ordinaires.' Hall and Nigrelli (25) reported in *Chlamydomonas* sp. the fusion of small elements of the vacuome to form larger globules approaching in size the 'vacuoles ordinaires' described by Dangeard. The vacuome of *Haematococcus pluvialis* (14) includes a number of globules scattered irregularly throughout the cytoplasm. A vacuome similar to that of other Phytomonadida has also been reported (11) in *Gonium*, *Eudorina* and *Volvox*.

It has been pointed out (19, 21) that the vacuome in plants serves as a center for the accumulation of various products of metabolism, especially those soluble in water, and thus should not be considered a part of the living substance but more properly one of the components of the paraplast, or deutoplasm. It might readily be assumed that the vacuome in the plant-like flagellates plays some such rôle in cell activities. This view is supported by

the positive reaction of the vacuome in *Chlamydomonas* (25) to the iodine test for starch.

GOLGI MATERIAL

Some years ago Bowen (2) stated, concerning the Golgi material of Protozoa, that with so many divergent opinions "no basis for what may or may not be Golgi material has yet been agreed upon." This statement still holds, since various types of inclusions have been identified by different workers as Golgi material of Protozoa. Several investigators have been tempted to recognize the vacuome of the plant-like flagellates as Golgi material. Others have been equally confident that the stigma, the contractile vacuole, or other specialized organelles should be considered Golgi material.

The similarities of the vacuome to Golgi material have been pointed out in a number of flagellates. In *Chromulina* sp. (23) small globules are blackened in osmic impregnation and resist bleaching with hydrogen peroxide; these inclusions resemble in size, shape and distribution the elements of the vacuome, and presumably are identical with the latter. *Chilomonas paramecium* (23) shows similar osmiophilic globules scattered through the cytoplasm; these likewise appear to be identical with the vacuome. In *Euglena gracilis* (24) small, scattered globules (vacuome) are impregnated consistently by osmic and silver methods, while the stigma is impregnated only occasionally and is much less resistant to bleaching after osmic impregnation than are the elements of the vacuome. Baker (1) has described the Golgi material of *E. gracilis* as follows: "These globules are spherical, oval or ring-shaped and invariably show an irregular inner surface of the periphery. They are quite similar in size to the globules making up the vacuome (volutin) which can be recognized in the same cells alongside these Golgi bodies as light-brown globules. The latter never take the osmic acid other than to appear as light vacuoles even in unbleached organisms. . . . It is believed that those globules which retain the black rim and inner gray center after Kolatchev's method with bleaching, are Golgi bodies and that these bodies are separate and distinct from the vacuome." It is not entirely certain, however, that Baker has succeeded in distinguishing between the vacuome and the scattered orange pigment granules

commonly observed in *Euglena gracilis*. In *Colacium vesiculosum* (28) the elements of the vacuome are consistently blackened in silver impregnation. *Astasia* sp. (24) shows small osmiophilic globules which appear to be identical with the vacuome; likewise, in *Menoidium incurvum* (24) both osmic and silver impregnation methods demonstrate small, scattered globules similar in size and distribution to the elements of the vacuome. In osmic impregnation of *Peranema trichophorum* (22) numerous small globules, apparently identical with the vacuome, are blackened consistently. In *Chlamydomonas* sp. (25) osmic and silver impregnation reveals a variable number of blackened globules, similar in relative size, number and distribution to the inclusions stained vitally with neutral red. In *Polytoma uvella* (34) the usual osmic and silver Golgi methods impregnate the elements of the vacuome, but not the contractile vacuole or the parabasal bodies. In *Haematococcus pluvialis* (14) scattered globules, apparently the vacuome, are blackened in osmic and silver impregnation.

In addition to the vacuome, various other organelles and inclusions have been designated Golgi material. Nassonov (32) maintained that the contractile vacuole of *Chilomonas paramecium* should be regarded as the homologue of the metazoan Golgi apparatus. In the dinoflagellate, *Polykrikos schwartzi* (7), clusters of elongated osmiophilic vesicles have been described around each centrosome; these inclusions have been considered Golgi material. Grassé (13, 15, 16, 17, 18) considers the stigma of *Euglena* homologous with the parabasal body of other flagellates and the equivalent of the Golgi apparatus. This interpretation has been questioned, particularly by Mangenot (31). Brown (3) has pictured the Golgi apparatus of *Peranema trichophorum* as "a network of long, interwoven fibres which are concentrated in the posterior portion of the animal. This network is not so dense in the later division stages of *Peranema* as it is during the early prophase." This 'Golgi network' described by Brown appears to be nothing more than the blackened pellicular striations of the flagellate, previously described (27) in various species as the 'silverline system' of *Euglenida*.

In attempting to recognize 'Golgi material' in Protozoa, it seems reasonable to look for inclusions with characteristics somewhat as follows: (1) consistently impregnated by the osmic methods, rather

than occasionally impregnated; (2) resistant to the usual methods of bleaching after osmication; (3) consistently impregnated by the silver methods; (4) except for possible specialized types, perhaps similar in general form in different Protozoa; (5) occurrence in Protozoa generally, and not merely in certain species or groups. If such criteria are applied to certain examples of so-called 'Golgi apparatus', the basis for their identification as Golgi material seems rather inadequate. On the other hand, the observations of a number of workers show that the vacuome more nearly satisfy such requirements than do any of the other types of inclusions or cell organelles previously designated as Golgi material. Hence, it would seem that recognition of the vacuome of flagellates as 'Golgi material' is at least as logical as attempting to identify such specialized structures as the stigma of *Euglena*, the parabasal body of certain flagellates, the contractile vacuole, or other organelles as homologues of the Golgi apparatus. However, this view is opposed by various workers who insist that, even in the green flagellates, the vacuome cannot be homologized with Golgi material. At present, therefore, the identity of the Golgi material in the Phytomastigoda is uncertain.

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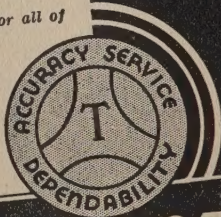
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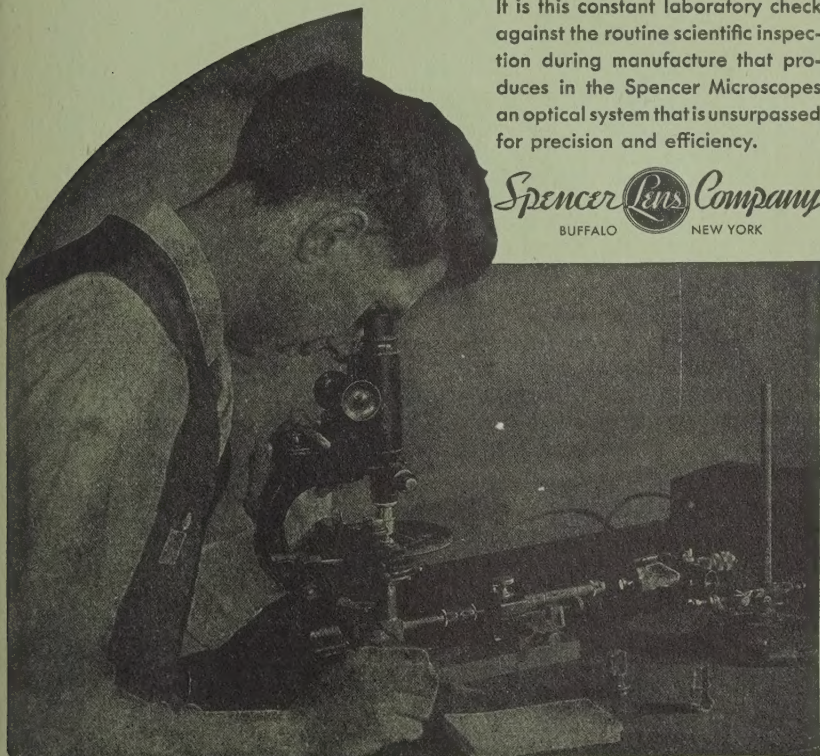
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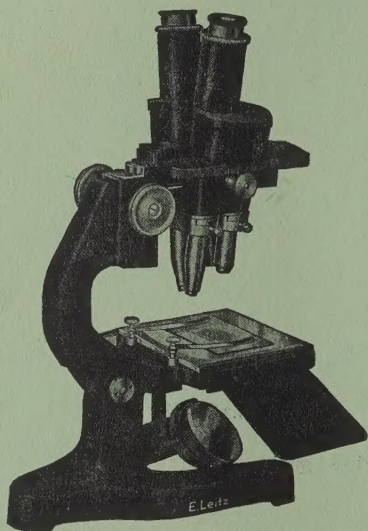
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